● **Original Article**

Temporary Effect of Hyperbaric Oxygen Therapy for Rat Focal Cerebral Ischemia

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The effect of hyperbaric oxygen (HBO) on the histopathological outcome of focal cerebral ischemia was investigated, using rats having middle cerebral artery occlusion (MCAO). Using halothane anesthesia, a 3-0 nylor suture was introduced through the extracranial internal carotid artery in order to occlude the left MCA. All rats received either 4-hr or 24-hr ischemia. Noncontrol animals were treated between 2.5-3.5 hrs following MCAO. HBO therapy was performed at 2 ATA with 100% oxygen. After perfusion fixation, either ischemic neuronal injury or infarction was examined by way of hematoxylin-eosin and Mallory-azan stainings. Four hrs following MCAO, treated animals showed a less % of infarct volume (18.1±9.7%) than non-treated controls (27.9±5.5% ; $p<0.01$). However, 24 hrs following MCAO, treated animals failed to show a significant decrease in infarct volume (28.0±7.4%), compared with controls (30.0±9.0%). In conclusion, HBO reduces ischemic neuronal injury as long as 4 hrs following MCAO in rats treated between 2.5-3.5 hrs after an ischemic insult. This therapeutic effect does not last for 24 hrs, however. Thus, the effect of HBO should be considered temporary.

Keywords :
Hyperbaric Oxygen Therapy
Focal Cerebral Ischemia
Rat
Histopathology
Temporary Effect

INTRODUCTION

Hyperbaric oxygen therapy (HBO) has always been considered as being a noninvasive means of treating cerebral ischemia and brain injury. Contreras et al[1] have demonstrated that improvements in glucose metabolism persist "at least 1 day" after the termination of HBO in a freeze-traumatized rat brain. However, whether the therapeutic effects of HBO continue is still controversial. In addition, there are few histopathological studies about the effects of HBO on cerebral ischemia. Therefore, we investigated HBO effects based on the histopathological outcomes of an improved rat model of focal cerebral ischemia[2].

Recently, Zea Longa et al[3] described a rat model of unilateral middle cerebral artery occlusion (MCAO) where an intraluminal suture technique was used. This technique can produce constant focal cerebral ischemia without any aid from hypotension, hypoxia, or hypovolemia. Moreover, it does not require a craniectomy, and therefore provides a simple and relatively non-invasive model. Previously,

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Table 1 Physiological Data during MCA Occlusion

Group(n)	MABP (mmHg)	P_aO_2 (mmHg)	P_aCO_2 (mmHg)	pH	Hct (%)	Total Blood or Plasma Glucose (mg/dl)
1 (10)	84±7	117±11	50±6	7.36±0.04	41±2	145±16(Blood)
2 (10)	82±13	116±10	50±5	7.36±0.02	41±1	146±12(Blood)
3 (10)	84±10	104±8	50±6	7.37±0.05	41±2	213±12(Plasma)
4 (10)	86±12	103±15	52±3	7.35±0.04	41±2	225±20(Plasma)
5 (6)	91±9	109±10	49±4	7.38±0.02	40±1	231±18(Plasma)
6 (11)	90±7	113±10	50±4	7.37±0.02	41±1	220±13(Plasma)

Values are mean±SD. n=animal number. See text for explanation of groups.

Table 2 Physiological Data immediately prior to Sacrifice

Group(n)	MABP (mmHg)	P_aO_2 (mmHg)	P_aCO_2 (mmHg)	pH	Hct (%)	Plasma Glucose (mg/dl)
1 (10)	98±9	116±5	48±5	7.34±0.04	43±2	not done
2 (10)	95±13	117±12	49±4	7.35±0.04	43±3	not done
3 (10)	98±11	114±8	46±4	7.41±0.03	44±3	137±11
4 (10)	102±20	115±5	46±6	7.41±0.05	46±2	145±11
5 (6)	99±8	118±5	46±2	7.41±0.01	45±2	148±12
6 (11)	101±15	113±11	48±7	7.40±0.05	44±2	152±19

Values are mean±SD. n=animal number. See text for explanation of groups.

a 4-0 nylon suture had been used as an intraluminal suture[3)~5)]. The suture of Zea Longa[3)] was made by rounding the tip only. Considering the diameter decrease from the internal carotid artery (ICA) to the anterior cerebral artery (ACA), a tapering suture (for insertion into the ACA) must be considered reasonable as an embolic source. Therefore, in this study, we used a 3-0 nylon suture with a tapered and rounded tip.

MATERIALS AND METHODS

1. Surgical technique

Male Sprague-Dawley rats (Charles River Japan, Inc.), weighing 250~350 g, were anesthetized with 1% halothane in a mixture of nitrous oxide/oxygen (70%/30%). The animals were allowed to breathe spontaneuosly. Rectal temperature was maintained at 37.5±0.1℃. The tail artery was cannulated for continuous blood pressure monitoring, and for blood sampling for the measurements of P_aO_2, P_aCO_2, pH, hematocrit, and total blood or plasma glucose. All of these physiological values during MCAO as well as prior to sacrifice, were similar for both control and treated animals **(Table 1, 2)**.

We occluded the left MCA as described previously[2)]. Briefly, the left external carotid artery (ECA), the ICA, and the pterygopalatine artery (PPA) were isolated in the neck **(Fig. 1a)**, and the PPA origin was occluded permanently with a microvascular clip **(Fig. 1b)**. The ECA was ligated and cut **(Fig. 1c)**, and the ECA stump was pulled downward **(Fig. 1d)**. A silk suture was tied around the

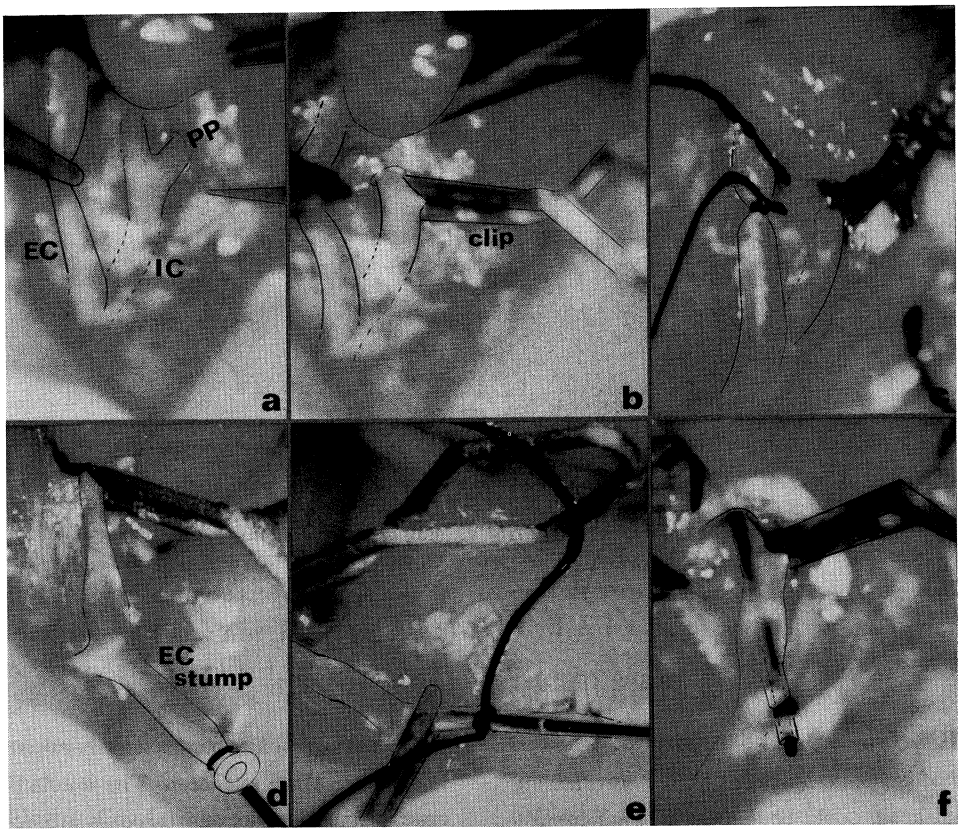

Fig. 1 Operative views during middle cerebral artery occlusion using the intraluminal suture technique
EC=external carotid artery, IC=internal carotid artery,
PP=pterygopalatine artery. See text for further explanation.

ECA stump, and a microvascular clip was placed temporally at the origin of the ECA stump. A 3-0 nylon suture was then introduced into the ECA stump **(Fig. 1e)**. The silk suture around the ECA stump was tightened to prevent bleeding, and the microvascular clip was removed. The nylon suture was advanced about 17.5mm into the ICA **(Fig. 1f)**, and the MCA origin was then occluded by the intraluminal suture. The catheter in the tail artery was filled with heparin, and heat-sealed. All animals were sacrified at either 4 hrs or 24 hrs following MCAO.

2. Histopathological Outcomes

Animals, respirating spontaneously, were anesthetized with pentobarbital (40 mg/Kg, i.p.). The rectal temperature was kept at 37.5± 0.1℃. Heparin (1000 IU/Kg, i.v.) was administered, and the tail artery again served for blood pressure monitoring and blood sampling. Perfusion fixation was performed with 10% formalin. Before the brains were removed from the skull, the skull base was removed to confirm the tip position of the intraluminal suture. The suture tip was found in the ACA in all of the cases.

Coronal sections (5μm thick) were stained with hematoxylin-eosin (HE) and Mallory-azan, and examined by light microscopy (40x, 200x). An infarct was seen within the occlud-

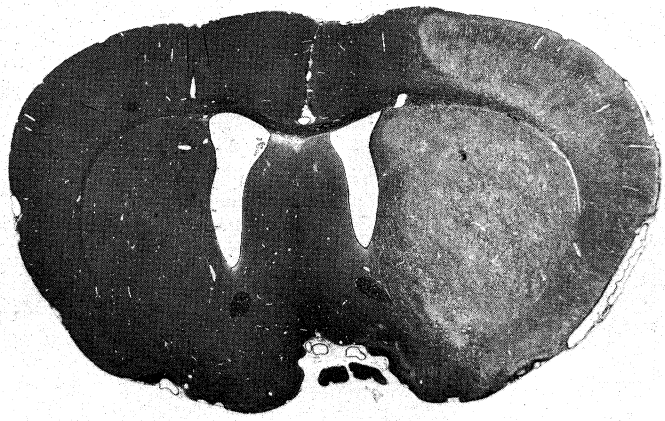

Fig. 2 Histopathology in a control animal 24 hrs following middle cerebral artery occlusion
HE stain.

ed MCA territory. The boundaries between areas of ischemic damage and the surrounding normal brain could be deliniated. Four hrs following MCAO, on the HE-stained sections, degenerating neurons became darkly stained, and had mildly atrophied cell bodies as well as triangular, shrunken nuclei. These cells are distinguishable from dark neuron artifacts. Surrounding the degenerating neurons, there was much microvaculation, indicating brain edema. Twenty-four hrs following MCAO, the infarct could be more easily distinguished from surrounding regions on the basis of gross cell loss and the appearance of generally disrupted tissue **(Fig. 2)**.

Area measurements were carried out at 8 coronal levels, chosen at 1.5 mm intervals. The first level was 2.5 mm posterior to the frontal tip. Areas of ischemic neuronal injury or infarct were plotted on tracing paper from projections of the coronal sections. The area of ischemic damage was then measured using an image analyzer (TAS, Leitz, FRG), and the infarct volume was calculated by computer. The volume of each hemisphere between the 8 levels was also calculated. The amount of ischemic damage was expressed as a percent of the total cerebral volume (% of infarct volume).

3. Experimental Protocol

Fifty-seven rats were used in this study. In the first experiment, Group-1 and -2 rats had 4hr-ischemia (10 rats/group). Group 1 served as a control group, and Group 2 received HBO therapy between 2.5 and 3.5 hrs following MCAO. Because a therapeutic effect was observed in the first experiment (see RESULTS), we underwent the second experiment. Group-3 and -4 rats had 24 hr-ischemia (10 rats/group). Group 3 served as a control group, and Group 4 received the same therapy as Group 2. In the third experiment, 24hr- ischemia was performed on Group 5 (6 rats) and Group 6 (11 rats). Group 6 was treated between 2.0 and 3.5 hrs following MCAO, and Group 5 served as its control group.

Treated animals were placed in a hyperbaric chamber (KHO-100, Kawasaki-Engineering,

Table 3 The Mean±SD Percent of Infarct Volume in Each Group

Group(n)	Treatment	Duration of ischemia	% of infarct volume	
1 (10)	Room-air	4 hrs	27.9±5.5	p<0.01
2 (10)	HBO(30 min*)	4 hrs	18.1±9.7	
3 (10)	Room-air	24 hrs	30.0±9.0	ns
4 (10)	HBO(30 min*)	24 hrs	28.0±7.4	
5 (6)	Room-air	24 hrs	36.6±6.9	ns, p<0.05
6 (11)	HBO(60 min*)	24 hrs	35.7±8.0	

n=animal number, *2 ATA, ns=not significant.

Japan) which was pressurized to 2 ATA for either 30 min (Group 2 and 4) or 60 min (Group 6), with 100 % oxygen (compression at 0.1 ATA/min ; decompression at 0.05 ATA/min). The oxygen inflow and outflow rates of the chamber were about 2 m^3/hr during HBO. Control animals were placed in normobaric room-air.

When the CO_2 content in the chamber was measured using a CO_2 Analyzer Type-ZFP5 (Fuji Electric Co., Ltd., Japan), it was 340 ppm before HBO, 250~275 ppm during 2ATA-HBO, and 320 ppm immediately following HBO. The CO_2 content in room-air ranged from 330~340 ppm. Therefore, the treated animals did not inhale 100% oxygen, while the (present) method used to introduce oxygen into the chamber is the same as that used for stroke patients in a one-man chamber.

4. Statistical Analysis

All data are expressed as the means±SD. For a comparison between the control and treated animals, the unpaired Student's t-test was used. A p value<0.05 was considered significant.

RESULTS

The first Experiment showed that Group 2 had significantly less infarct size than Group 1 ($p<0.01$). However, the second experiment showed no significant decrease in infarct size in Group 4, when compared with Group 3. The HBO therapy having a 30 min longer duration was initiated 30 min earlier than the first and second experiments. In the third experiment, however, we detected no significant decrease in the infarct volume in Group 6, when compared with Group 5 **(Table 3)**.

DISCUSSION

HBO is considered to increase the tissue oxygen supply, resulting in an improved oxidative metabolism. Cerebral ischemia may be attenuated by this treatment, whereas vasoconstriction due to hyperoxemia induces a decrease in the cerebral blood flow (CBF)[6)~8)]. The CBF decrease, as well as vasoconstriction, can induce a decrease of cerebral blood volume (CBV), resulting in a decrease of intracranial pressure (ICP). Therefore, in spite of the CBF decrease, both the oxygen-supply increase and the ICP decrease can improve the brain ischemia.

The first experiment in this study demonstrates that, within 4 hrs after MCAO, HBO therapy reduced the ischemic neuronal injury in rats treated between 2.5 and 3.5 hrs following an ischemic insult. In clinical settings, HBO therapy has been performed for stroke patients "once a day." When the stroke patients receive their therapy, however, we should consider whether the therapeutic effects of HBO continue for at least 24 hrs.

In the second experiment, we found that the

therapeutic effects observed in the first experiment had ceased within 24 hrs following MCAO. Thus, the HBO effect on cerebral ischemia must be considered temporary. Neither could the therapeutic effect of HBO be demonstrated by the third experiment, even when the therapy was started earlier and had a longer duration. On the contrary, the animals that received the longer therapy (Group 6) had a larger infarct size than those that received the shorter therapy (Group 4; $p < 0.05$), while the infarct sizes in control groups (Groups 3 and 5) showed no significant difference.

The threshold for cellular damage may explain the mechanism responsible for the temporary effect of HBO. This threshold should be determined by the severity and duration of ischemia[9)10)]. The results of Jones et al[9] suggest an infarction threshold, rising over several hours to a plateau at a CBF value of 17~18 ml/100g/min, below which normal brain tissue is irreversibly damaged. We posturate that, even though HBO can delay an ischemic neuronal injury until 4 hrs following an ischemic insult, the next 20 hrs may be enough to induce the same outcomes from ischemia as found in non-treated controls. It is unlikely that HBO alone is sufficient to treat cerebral ischemia. However, if HBO therapy is used in conjunction with other therapeutic measures on stroke patients, it is possible that that treatment may have a favorable effect.

In conclusion, for at least 4 hrs following MCAO, HBO therapy reduces ischemic neuronal injury in rats that are treated between 2.5 and 3.5 hrs following MCAO. However, this favorable effect does not persist for 24 hrs. Based on our observations in the histopathological study, the therapeutic effect of HBO should be considered temporary.

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[REFERENCES]

1) Contreras FL, Kadekaro M, Eisenberg HM: The effect of hyperbaric oxygen on glucose utilization in a freeze-traumatized rat brain. J Neurosurg 68: 137-141, 1988
2) Kawamura S, Yasui N, Shirasawa M, Fukasawa H: Rat middle cerebral artery occlusion using an intraluminal thread technique. Acta Neurochir 109: 126-132, 1991
3) Zea Longa E, Weinstein PR, Carlson S, Cummins R: Reversible middle cerebral artery occlusion without craniectomy in rats. Stroke 20: 84-91, 1989
4) Koizumi J, Yoshida Y, Nakazawa T, Ooneda G: Experimental studies of ischemic brain edema 1. A new experimental model of cerebral embolism in rats in which recirculation can be introduced in the ischemic area. Jpn J Stroke 8: 1-8, 1986
5) Nagasawa H, Kogure K: Correlation between cerebral blood flow and histologic changes in a new rat model of middle cerebral artery occlusion. Stroke 20: 1037-1043, 1989
6) Sukoff MH, Hollin SA, Espinosa OE, Jacobson JH: The protective effect of hyperbaric oxygenation in experimental cerebral edema. J Neurosurg 29: 236-241, 1968
7) Hart GB, Thompson RE: The treatment of cerebral ischemia with hyperbaric oxygen (OHP). Stroke 2: 247-250, 1971
8) Nakajima S, Meyer JS, Asano T, Shaw T, Okabe T, Morter KF: Cerebral vasomotor responsiveness during 100% oxygen inhalation in cerebral ischemia. Arch Neurol 40: 271-276, 1983
9) Jones TH, Morawetz RB, Crowell RM, Marcoux FW, FitzGibbon SJ, DeGirolami U, Ojemann RG: Thresholds of focal cerebral ischemia in awake monkeys. J Neurosurg 54: 773-782, 1981
10) Weinstein PR, Anderson GG, Telles DA: Neurological deficit and cerebral infarction after temporary middle cerebral artery occlusion in unanesthetized cats. Stroke 17: 318-324, 1986

●原　著

嚢胞様黄斑浮腫に対する高気圧酸素療法

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網膜静脈閉塞症（RVO），白内障嚢外摘出＋レンズ挿入術（ECCE＋IOL）および糖尿病性網膜症（DR）に合併した嚢胞様黄斑浮腫（CME）合計19症例24眼に対し高気圧酸素療法（HBO）を施行した。

HBOは2 ATA下で100%酸素吸入1時間とした。約2週間にわたる1日1回のHBO終了直後では，RVOおよびECCE＋IOLに合併したCME12症例の67%，DRに合併したCME12眼（7症例）の75%で矯正視力の2段階以上の改善が得られた。

HBO後の観察期間（1～9カ月間）を含めるとRVOおよびECCE＋IOLでは1症例を除き全例で2段階以上の改善が得られた。しかし，DRでは有効率は75%より33%に低下した。

CMEに対するHBOの視機能改善の機序は不明であるが，以上の結果よりRVOおよびECCE＋IOLに合併したCMEに対してはHBOは有効な治療法と考えられた。

キーワード：高気圧酸素療法，嚢胞様黄斑浮腫，網膜静脈閉塞症，糖尿病性網膜症，眼内手術

The effect of hyperbaric oxygen therapy on cystoid macular edema

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Twenty-four eyes of 19 cases with established cystoid macular edema (CME) secondary to retinal vein occlusion (RVO), post-intraocular sugery of extracapsular cataract extraction and intraocular lens (ECCE＋IOL), and diabetic retinopathy (DR) were treated with hyperbaric oxygen.

Hyperbaric oxygen therapy (HBO) was instituted on patients breathing humidified 100% oxygen at 2.0 ATA for one hour.

Immediately after daily HBO for about 2 weeks, visual acuity improved by two or more Snellen lines in 67 % of 12 CME cases secondary to RVO and ECCE＋IOL, and in 75% of 12 CME eyes (7 cases) with DR.

After the follow-up period of 1-9 months, visual acuity improved effectively, except one case with RVO, in all cases secondary to RVO and ECCE＋IOL. In the cases with DR, however, visual acuity has tended to regress with time.

Although the mechanism of HBO to improve visual function in CME is unclear, these results suggest that HBO would be effective in CME secondary to RVO or post-intraocular surgery.

Keywords:
Hyperbaric oxygen therapy
Cystoid macular edema
Retinal vein occlusion
Diabetic retinopathy
Post-intraocular surgery

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